Water for sterol: a novel mechanism of sterol egress from the binding pocket of a yeast StARkin domain

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Proteins with steroidogenic acute regulatory protein related lipid transfer (StART) domains (the StARkin superfamily [1]) are implicated in intracellular, non-vesicular lipid transport. A family of endoplasmic reticulum membrane-anchored StARkins was recently identified, including six members Lam1-Lam6 in *Saccharomyces cerevisiae.* Lam1-Lam4 localize to ER-plasma membrane contact sites, where they play a role in sterol homeostasis [2]. We recently reported crystal structures of the second StARkin domain of Lam4 (here termed Lam4S2) in the *apo*- and sterol-bound states [3]. We found that the binding pocket of the protein was surprisingly open: whereas bound sterol was shielded at the site of entry/exit from the binding pocket, it was unexpectedly solvent-exposed along the side of the pocket. We considered whether the energetically costly exposure of the sterol backbone to solvent could play a role in sterol egress from the binding site by allowing water molecules to enter/exit the pocket, influencing the stability of the bound sterol.

We used a combination of large-scale atomistic molecular dynamics (MD) simulations and functional experiments to test this hypothesis. Analysis of extensive (>40 μs) ensemble and umbrella sampling MD trajectories revealed a possible mechanism for the spontaneous release of bound sterol into the membrane. The simulations revealed that sterol egress is triggered by widening of the side-entrance to the binding pocket, penetration of water molecules into the cavity and consequent destabilization of the bound sterol. We identified several polar residues that line the side-entrance to the pocket and that play a critical role in the initial steps of the release process. The functional importance of these residues was validated experimentally by showing that their replacement with Ala affected the ability of Lam4S2 to alleviate the nystatin-sensitivity of a *lam2Δ* strain and reduced the efficiency with which the purified protein is able to catalyze inter-vesicular transport of sterol in an *in vitro* assay. These data suggest an unprecedented and non-intuitive mechanism of sterol acquisition and discharge from a StART domain.

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